EVIDENCE FOR A PING-PONG MECHANISM IN THE DIAMINE OXIDASE REACTION

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1. Introduction

Diamine oxidase from pig kidney, an enzyme which contains copper and pyridoxal phosphate [1–2], deaminates oxidatively diamines yielding the corresponding aldehydes, ammonia and hydrogen peroxide. In this paper we shall report evidence for the anaerobic release of the aldehyde and suggest a ping-pong mechanism for the reaction.

2. Materials and methods

The enzyme was purified and assayed as described elsewhere [1,2]. Molar concentrations of enzyme are expressed on the basis of a minimum molecular weight of 90,000 calculated from the copper content [1]. Cadaverine 1–5 ^{14}C hydrochloride (3.3 Curie/Mole) was from New England Nuclear Corp., Boston, Mass. The piperidine derivative of the amino-aldehyde product was extracted from reaction mixtures according to Okuyama and Kobayashi [3]. Radioactivity was measured with a Nuclear Chicago Liquid Scintillation Counter Mod. 725.

The vials for scintillation counting were prepared as described elsewhere [4]. The efficiency was calculated by a channel ratio technique.

Steady state kinetics experiments were performed with a Gilson Medical Electronic Oxygraph. Anaerobiosis was performed according to Malmström et al. [5].

3. Results and discussion

2.5×10^{-5} M diamine oxidase was incubated in 2 ml of 0.1 M phosphate buffer pH 7.4 in the absence of oxygen with 3×10^{-4} M labelled cadaverine for 30 min at 38°. After this time the reaction was stopped by adding trichloroacetic acid anaerobically. Control experiments were performed with labelled substrate alone. The trichloroacetic treated samples were then exposed to air and extracted with 5×3 ml toluene. The toluene extracts were collected and subjected to scintillation counting. Calculation of the radioactive product:enzyme ratio gave a value of 0.9 moles of labelled piperidine per mole of enzyme. A comparable result has recently been obtained with other amine oxidases [6].

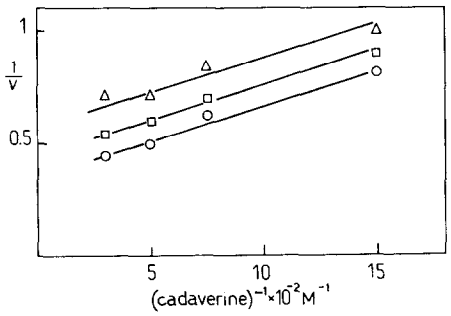

Fig. 1. Lineweaver-Burk plots of diamine oxidase velocities with cadaverine as variable substrate. ○–○ in the presence of 17×10^{-5} M oxygen; □–□ in the presence of 4.25×10^{-5} M oxygen; △–△ in the presence of 2.13×10^{-5} M oxygen.

This result suggests that the diamine oxidase reaction could be described according to a mechanism in which the enzyme reacts with the amine substrate first through its pyridoxal phosphate moiety, yielding the aldehyde product. The reduced enzyme reacts then with oxygen to release the final products NH_3 and H_2O_2. Such a mechanism in which the enzyme reacts in turn with each substrate (i.e. amine and oxygen) via a binary complex could be easily verified by a simple steady-state kinetics experiment. In fact, for a mechanism of this kind, plots of reciprocal velocity versus reciprocal concentrations of one substrate (variable substrate) are parallel lines of identical slope, regardless of the concentration of the fixed substrate. Fig. 1 shows that experiments of this type gave results which fit in fairly well with such a mechanism.

A ping-pong mechanism has been demonstrated for other pyridoxal phosphate-containing enzymes [7]. The fact that a similar scheme of reaction can be applied to diamine oxidase also supports the idea of an essential role of this coenzyme for the catalytic action of diamine oxidases. Further study is required to demonstrate in a more direct way the involvement of pyridoxal phosphate in a catalytic step of the reaction, and to elucidate the role of copper in the overall catalytic sequence.

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